Cell Host & Microbe
In Translation

The Quest for a Universal Flu Vaccine: Headless HA 2.0

Florian Krammer[1,*]
[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
*Correspondence: florian.krammer@mssm.edu
http://dx.doi.org/10.1016/j.chom.2015.10.003

Two recent publications report the design of immunogens based on the conserved stalk domain of the influenza virus hemagglutinin. These new "headless" hemagglutinin constructs recapitulate the epitopes recognized by broadly neutralizing antibodies and induce broadly protective/heterosubtypic immunity in animal models, bringing us a step closer to "universal" influenza virus vaccines.

Influenza viruses escape human herd immunity by continuously changing their surface antigens, a process called antigenic drift. Due to this antigenic drift influenza virus vaccines have to be reformulated and re-administered on an annual basis. The discovery of broadly neutralizing antibodies against the relatively conserved stalk domain of the viral hemagglutinin (HA) in recent years has spurred the development of "universal" influenza virus vaccines. Unfortunately, however, it is difficult to induce antibodies against this domain with standard vaccines due to the immunodominance of the membrane distal globular head domain of the HA, which is the region most affected by antigenic drift.

The immunosubdominance of the stalk domain has been an obstacle for the development of broadly protective stalk-based vaccines. The seemingly obvious solution to this problem is the removal of the immunodominant globular head domain resulting in a stalk-only or headless HA. The first approach toward a headless HA was reported by Graves et al. (1983) (Table 1). By exposing influenza virions to acidic and reducing conditions it was possible to remove the HA1 subunit (which includes the whole head domain) and generate virions with an exposed HA2 subunit (which includes most of the stalk domain). At that point, it was not clear that broadly neutralizing anti-stalk antibodies bind to conformational epitopes that require correctly folded pre-fusion stalk domains. In fact, the chemical treatment of the virions had destroyed those fragile epitopes. Furthermore, the N- and C-terminal parts of the HA1—which were completely removed by this approach—are part of the stalk domain, as well. Studies by (Sagawa et al. (1996) and Steel et al. (2010) genetically removed the globular head domain. These vaccine constructs proved to have protective potential in the mouse model, but the removal of the globular head domain most likely led to the misfolding of the resultant headless HA immunogens and concomitant destruction of important neutralizing epitopes. Bommakanti et al. (2012) reported an alternative version of a headless HA expressed in *E. coli*. While this construct was protective against mortality from a heterologous virus challenge, its conformation was likely far from optimal. A follow up study by (Mallajosyula et al. (2014) reported enhanced structural integrity and improved efficacy in the mouse model. Another recent study by Lu et al. (2014) involved a multistep rational design approach and led to a construct that displayed correctly folded conformational epitopes of broadly neutralizing antibodies. However, no reports of the in vivo efficacy of this immunogen were published. Finally, Wohlbold et al. (2015) showed that a soluble recombinant headless HA produced in insect cells provided protection against lethal H1N1, H5N1, and H6N1 challenges despite the fact that the immunogen was not folded correctly.

In two very elegant recent studies Impagliazzo et al. (2015) and Yassine et al (2015) independently reported methods for making stable, correctly folded headless HA molecules (Figure 1; Table 1). While both studies take different paths to success, they share common design elements. Previous headless HA approaches focused on stabilizing the molecule by adding a "clamp" (usually a trimerization domain) at the membrane proximal end of the molecule. This approach initially appeared sensible, as this portion of the molecule is normally stabilized by the transmembrane domain in virion-associated HA. However, this design does not improve the stability of the membrane-distal part of the headless HA, which exposes a relatively hydrophobic surface that is usually hidden below the head domain. The introduction of a stabilizing element in this region led to success for both new approaches. Instead of introducing a trimerization domain at the membrane distal end of the HA, Impagliazzo et al. chose to replace the upper part of the central long alpha helix of HA2 with a leucine zipper trimerization domain (which also forms a helix). Similarly, Yassine et al. fused an HIV gp41 trimerization domain to the upper part of the long alpha helix. While Yassine et al. removed this design element from the final version of their immunogen, the introduced leucine zipper remains as an important stabilizer in the Impagliazzo headless HA. In addition, both groups used the stalk domains from pre-pandemic H1N1 strains as the framework for the headless HA design. This framework might have contributed to success since the stalk domain from 2009 pandemic H1N1 viruses is less stable. Structural characterization and binding analysis with broadly neutralizing anti-stalk monoclonal antibodies (mAbs) provide evidence of the structural integrity of both constructs. Importantly, both constructs induce stalk-reactive antibodies in animals and protect against heterosubtypic challenge with highly pathogenic H5N1 viruses. Interestingly, this protection was achieved in the absence of robust neutralizing antibody titers. However, stalk-reactive antibodies are known to protect through several mechanisms including inhibition of entry, inhibition of viral egress, inhibition of HA maturation and via effector functions

Table 1. Summary Table of Group 1 Headless HA Studies

Study	HA Basis	Platform/Construct	Homologous protection	Heterosubtypic protection	Stalk mAb binding
Graves et al. (1983)	H1 (A/PR/8/34) and others	Virus particles	ND[1]	ND	ND, unlikely
Sagawa et al.(1996)	H2 (A/Okuda/57)	Transfected cells	ND	H1N1: 70%[2]	mAb C179 binding confirmed by immunostaining
Steel et al. (2010)	H1 (A/PR/8/34) and others	Virus-like particles	Yes[2]	ND	No, as shown by Impagliazzo et al.
Bommakanti et al. (2012)	H1 (A/PR/8/34), (A/NC/20/99), (A/California/07/09)	Bacterial expression of soluble protein	Yes[2]	ND	mAb CR6261 binding by surface plasmon resonance (SPR)
Lu et al. (2014)	H1 (A/California/05/09)	Cell free synthesis of soluble protein	ND	ND	mAb CR6261, FI6 and C179 binding in capture ELISA
Mallajosyula et al. (2014)	H1 (A/PR/8/34)	Bacterial expressed soluble protein	Yes[2]	H3N2: 30%[2] (control: 10%)	mAb CR6261, FI6 and F10 binding by SPR
Wohlbold et al. (2015)	H1 (A/PR/8/34)	Insect cell expressed soluble protein	Yes[2]	H5N1: 60%[2] H6N1: 100%[2]	No binding with panel of stalk mAbs in direct coating ELISA
Impagliazzo et al. (2015)	H1 (A/Brisbane/59/07)	Mammalian cell expressed soluble protein	Yes[2] Non-human primates: reduced fever	H5N1: 100%[2]	Binding of CR9114 and CR6261(ELISA, structural methods, SPR)
Yassine et al. (2015)	H1 (A/NC/20/99)	Mammalian expressed ferritin nanoparticles	ND	H5N1: 100%[2] Ferrets H5N1: 67%	Binding of a panel of anti-stalk mAbs (ELISA, structural methods, SPR)

[1]Not determined.
[2]A as tested in the mouse model.

like antibody dependent cell-mediated immunity (ADCC) and complement dependent cytotoxicity. High levels of in vitro neutralizing antibodies might therefore not be required for robust stalk-based protection.

The construction of stable headless HA constructs is an important step toward the development of a universal influenza virus vaccine. However, there are several caveats associated with this approach. Both constructs include heterologous elements that raise concerns about autoimmunity. Leucine zipper motifs, such as those used in the Impagliazzo construct are common in nature and proteins with very similar sequences exist in humans. The bacterial ferritin scaffold used by Yassine and colleagues is highly immunogenic. The primary sequences of bacterial and mammalian ferritins show little sequence identity but a high degree of structural similarity exists between them. While the authors show in earlier studies that these ferritin scaffolds do not induce autoimmunity in inbred mice (Kanekiyo et al., 2013), humans who are predisposed to autoimmune disease may react differently. In addition, the strong immunogenicity (immunodominance) of the scaffold might reduce the immune response against the subdominant HA stalk domain. Furthermore, headless

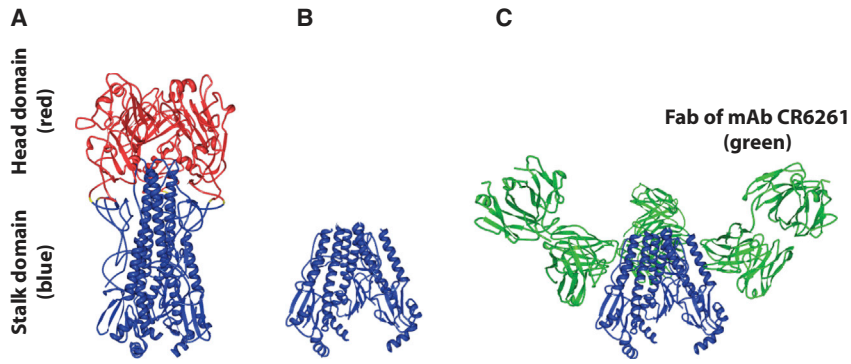

Figure 1. Novel Headless HA Constructs and Overview of Headless HA Studies Based on Group 1 Hemagglutinins
(A) Structure of full length A/California/04/09 H1 HA based on PDB# 4M4Y. The membrane proximal stalk domain is indicated in blue, the membrane distal globular head domain is shown in red. Cysteines 52 and 277 (H3 numbering) which form the demarcation line between the head and the stalk domain are shown in yellow.
(B) Structure of one of the intermediated stalk constructs (generation 4) from Yassine et al. The structure is based on PDB# 5C0S.
(C) The headless HA construct from (B) bound by Fab fragments of broadly neutralizing anti-stalk mAb CR6261 (green). Of note, the gp41 trimerization domain present in PDB 5C0S is not shown in (B) and (C) to simplify the concept.

HA constructs expose regions of the HA stalk, which are usually not accessible to the humoral immune system. It is therefore possible that a large proportion of antibodies induced by these headless HA constructs are unable to neutralize—or even bind—to native HA spikes on virions. Finally, both headless HA constructs are based on the stalk of an H1 HA, which belongs to group 1 HAs. In order to protect against currently circulating drift variants, a group 2 stalk-based headless HA as well as an influenza B stalk-based headless HA would be needed in a universal influenza virus vaccine formulation. It is unclear if designs similar to those used by Impagliazzo et al. (2015) and by Yassine et al. (2015) would also lead to stable group 2 and B headless HAs or if novel unique construct designs are needed. Although many open questions remain, the successful design of such elegant headless HA constructs (Impagliazzo et al., 2015; Yassine et al., 2015) is an important advancement in the direction of a truly universal influenza virus vaccine.

ACKNOWLEDGMENTS

FK..'s work on heterosubtypic influenza virus immunity is funded by National Institute of Health grants and contracts (U19 AI109946, R56 AI117287 and CEIRS HHSN272201400008C). The Krammer laboratory also receives funding from GlaxoSmithKline.

REFERENCES

Bommakanti, G., Lu, X., Citron, M.P., Najar, T.A., Heidecker, G.J., ter Meulen, J., Varadarajan, R., and Liang, X. (2012). J. Virol. *86*, 13434–13444.

Graves, P.N., Schulman, J.L., Young, J.F., and Palese, P. (1983). Virology *126*, 106–116.

Impagliazzo, A., Milder, F., Kuipers, H., Wagner, M.V., Zhu, X., Hoffman, R.M., van Meersbergen, R., Huizingh, J., Wanningen, P., Verspuij, J., et al. (2015). Science *349*, 1301–1306.

Kanekiyo, M., Wei, C.J., Yassine, H.M., McTamney, P.M., Boyington, J.C., Whittle, J.R., Rao, S.S., Kong, W.P., Wang, L., and Nabel, G.J. (2013). Nature *499*, 102–106.

Lu, Y., Welsh, J.P., and Swartz, J.R. (2014). Proc. Natl. Acad. Sci. USA *111*, 125–130.

Mallajosyula, V.V., Citron, M., Ferrara, F., Lu, X., Callahan, C., Heidecker, G.J., Sarma, S.P., Flynn, J.A., Temperton, N.J., Liang, X., and Varadarajan, R. (2014). Proc. Natl. Acad. Sci. USA *111*, E2514–E2523.

Sagawa, H., Ohshima, A., Kato, I., Okuno, Y., and Isegawa, Y. (1996). J. Gen. Virol. *77*, 1483–1487.

Steel, J., Lowen, A.C., Wang, T.T., Yondola, M., Gao, Q., Haye, K., García-Sastre, A., and Palese, P. (2010). MBio *1*, 1.

Wohlbold, T.J., Nachbagauer, R., Margine, I., Tan, G.S., Hirsh, A., and Krammer, F. (2015). Vaccine *33*, 3314–3321.

Yassine, H.M., Boyington, J.C., McTamney, P.M., Wei, C.J., Kanekiyo, M., Kong, W.P., Gallagher, J.R., Wang, L., Zhang, Y., Joyce, M.G., et al. (2015). Nat. Med. *21*, 1065–1070.